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TITLE: T-CELL SUBSETS IN PERIPHERAL BLOOD IN UVEITIS

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Purpose To investigate the peripheral blood T-cell subpopulations in patients with different forms of uveitis.
Methods The CD 3+, CD 4+ and CD 8+ lymphocyte subsets were studied in peripheral blood by flowcytometry in 220 patients with anterior, intermediate, posterior and panuveitis (150 cases with proven etiology and underlined clinical syndrome and 70 with idiopathic uveitis). The control group consisted of 30 healthy subjects..
Results The CD 4/CD 8 index was increased in 50% of patients with anterior uveitis, associated with systemic diseases, as it was seen in intermediate uveitis, while the idiopathic forms of the two groups did not exhibit any changes. The CD 8+ cells in active stage of toxoplasmic retinochoroiditis and the CD 4+ cells in TBC uveitis were significantly increased, compared to healthy controls. In almost all cases with Behcet's disease we found a decreased number of pan-T lymphocytes and disturbances mostly in CD 8+ cells.
Conclusions: A T-cell immune disbalance in peripheral blood was found predominantly in patients with uveitis, associated with diseases, accompanied by systemic immune manifestations.

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IMMUNOHISTOCHEMICAL DISTRIBUTION OF FK506 BINDING PROTEIN IN HUMAN OCULAR TISSUES.

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Purpose: FK506 binding protein-12 (FKBP-12), the 12-KDa. intracellular receptor for the immunosuppressive drug FK506 is widely distributed in eukaryote cells, but its localization in ocular tissues has not been investigated. Therefore, the distribution of FKBP-12 in ocular tissues was examined using immunohistochemical technique.
Methods: Human eye ball was obtained from the autopsy in Juntendo University Hospital. Two kinds of monoclonal antibodies (2C1 and 3F4) which have different epitopes of FKBP-12 were given by Fujisawa Pharmaceutical Co. Ltd.. The eye ball was divided into several pieces and were snap-frozen with liquid nitrogen. The frozen tissues were cut into 5μm sections and fixed in acetone. Then, sections were incubated with the antibodies 2C1 or 3F4 to FKBP-12. Immunohistochemical study was done using a horseradish peroxidase method.
Results: Positive reactions were strongly detected with both antibodies in corneal epithelium. But the positive staining of the antibody 2C1 seemed to localize in the nucleus and that of the antibody 3F4 was observed to localize in the cytoplasm of the corneal epithelium. Weak reactions were also detected with the antibody 2C1 in the nucleus of corneal keratocytes. On the other hand, the cytoplasm of the non-pigmented epithelium of the ciliary body was strongly stained with both antibodies. No reaction was observed to anti-FKBP-12 antibodies in iris and trabecular meshwork.
Conclusion: It was found that FKBP-12 is present in many cells of ocular tissues. However, we could not explain why antibody 2C1 localizes in the nucleus and 3F4 localizes in the cytoplasm of the corneal epithelium. Further investigation is needed to explain these findings.

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ENHANCED IN VIVO GENE TRANSFER INTO OCULAR TISSUES

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Purpose. To develop techniques to deliver more efficiently a recombinant adenoviral vector into ocular tissues of rabbits in vivo.
Methods. A replication-deficient adenoviral vector containing a marker gene was injected intracamerally, intravitreally and into the subretinal space. The injections were preceeded by lensectomy, vitrectomy or a combination of both procedures in order to optimize the diffusion of the adenoviral vector. Titers ranging from 10^7 to 10^{10} pfu were delivered, and the eyes were analysed 7 to 52 days after injection using histochemical in situ β-galactosidase activity.
Results. Intense transgene expression was observed in the corneal endothelial cells, and the trabecular meshwork for the titers up to 10^{10} pfu with no toxic effect. Intravitreal injection combined to vitrectomy increased the expression of the adenoviral vector only for titers higher than 108 pfu. In posterior segment injections only exposed cells to the adenoviral vector displayed a blue staining. Photoreceptor cells are infected exclusively in subretinal injections with high titers of vectors and with no cytopathic effect.
Conclusions. Multiple subretinal injections might be required in gene transfer strategies targeting the photoreceptor cell layer.

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TITLE: EFFICIENT AND NONINVASIVE IN VIVO GENE TRANSFER TECHNIQUE INTO ADULT MAMMALIAN OCULAR TISSUE

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Purpose We determined whether a reporter gene can be introduced into adult mammalian ocular tissue in vivo by the Sendai virus (hemagglutinating virus of Japan, HVJ)-liposome technique.
Methods HVJ-liposome complex containing a expression vector plasmid DNA of cytomegalovirus-promoted bacterial β-galactosidase (LacZ) were prepared. It was microinjected into anterior chamber, the vitreous, and subretinal space of adult Balb-c mice. LacZ expression was assessed by β-galactosidase assay.
Results LacZ expression was observed at 5 days after injection and most intense at 10-20 days. It was detected depending upon the injection site; iris-ciliary body (I-CB) in anterior chamber-injection animals, retina and I-CB in the vitreous-injection, and photoreceptors and retinal pigment epithelium in subretinal space-injection. No evidence indicating toxic effects was observed.
Conclusions These results demonstrate that HVJ-liposome can efficiently and safely mediate transfer of a foreign gene into adult mammalian ocular tissue in vivo.